* NOTICES *

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

Technical field

This invention relates to oxidation stress inhibitor useful as medicine for the therapy of an oxidant stress disease, and/or prevention. More particularly, this invention relates to the oxidation stress inhibitor which contains a pyrazolone derivative or its salt permitted pharmacologically as an active principle. Furthermore, this invention relates to the measuring method of oxidant stress. More particularly, this invention relates to the measuring method of the oxidant stress using mono-unsaturated fatty acid, the ubiquinone 10 (CoQ-10 is said), or cholesterol ester hydroperoxide in plasma as a marker.

Background art

If imbalance arises in an oxidation defense mechanism in the living body by a certain cause around intracellular or a cell, a biomembrane will oxidize, but what oxidizes easiliest is a higher unsaturated fatty acid in membrane lipids. In order to compensate reduction in a higher unsaturated fatty acid, a cell activates a fatty acid unsaturation enzyme and change to palmitoleic acid (16:1) of pulmitic acid (16:0) to oleic acid (18:1) of stearic acid (18:0) advances. When oxidant stress furthermore rises and a cell dies, hydrolase will work and fatty acid of isolation will come out into a blood flow, but. Compared with the case where the presentation of the free fatty acid has not required oxidant stress, it is known that there are few rates of a higher unsaturated fatty acid with many [rates] rates of mono-unsaturated fatty acid (oleic acid (18:1), palmitoleic acid (16:1)).

When generation of active oxygen and a free radical increases not only sthenia of the oxidant stress in a cell but in a blood flow, the antioxidants which decrease in number first are vitamin C and the ubiquinol 10. As for the ubiquinol 10, it is useful to make this into the index of oxidant stress in order to oxidize to the ubiquinone 10 selectively. After vitamin C and the ubiquinols 10 decrease in number, oxidation of the cholesterol ester in a lipoprotein advances and generation of the cholesterol ester hydroperoxide which is the oxidation product becomes remarkable. therefore, the antioxidant with this hydroperoxide useful as a second-half marker for oxidative stress which does not come to see but controls this generation has a practically big meaning.

As a disease (an "oxidant stress disease" may be called hereafter) which induces, or advances and gets worse by oxidant stress, a hepatopathy, cerebrovascular disease (cerebral infarction, cerebral hemorrhage, etc.), diabetes mellitus, etc. are mentioned. As an example for which the place by the present and mono-unsaturated fatty acid in plasma go up, there is a report by rat liver injury model (carbon tetrachloride

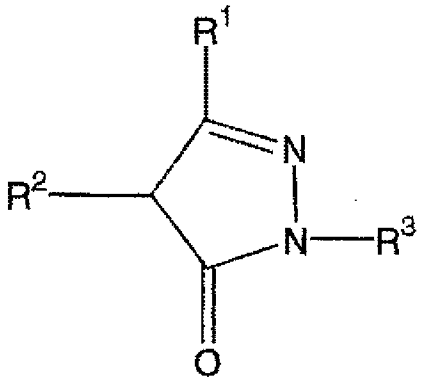
administration, LEC rat), cerebrovascular-disease acute stage patient, a newborn infant, etc. However, there is no report of the drugs which have the operation which reduces mono-unsaturated fatty acid in plasma. Although the oxidation obstacle in the fault part is recognized from the former, there is no report of having proved exactly and quantitatively the oxidation obstacle (for example, brain tissue receives an oxidation obstacle in cerebral infarction) in the fault part. Although a target organ is extracted and extracted by an animal experiment and methods, such as quantifying peroxylipid, are known, organization extraction is not realistic when evaluating the oxidation obstacle in organizations (for example, liver, a brain, etc.) at the clinical spot. The necessity of developing the method of measuring blood etc. as a preparation, without carrying out invasion of the organization for an oxidation obstacle exactly and quantitatively still exists.

The indication of an invention

This invention made it the issue which should be solved to provide oxidation stress inhibitor useful as medicine for the therapy of a safe oxidant stress disease with few side effects, and/or prevention. This invention made it the issue which should be solved to provide the new method for measuring oxidant stress exactly and quantitatively again. It made for this invention to provide the measuring method of new oxidant stress which can measure blood etc. as a preparation again without carrying out invasion of the organization into the issue which should be solved. This invention made it the issue which should be solved to provide the method of evaluating the validity of the oxidant stress depressant action which the pyrazolone derivative which has a fixed structure written in this Description has.

As a result of this invention persons' inquiring wholeheartedly in order to solve an aforementioned problem, and measuring change of the marker for oxidative stress (mono-unsaturated fatty acid in plasma, i.e., oleic acid, (18:1), palmitoleic acid (16:1)) in a rat brain ischemia-recanalization model, the rise of the concentration was accepted. When the pyrazolone derivative shown by following formula (I) which this Description defines as a result of examining whether this oxidant stress can be controlled by administration of a drug was prescribed for the patient, it found out that a rise of the marker for oxidative stress mentioned above could be controlled. Also when change of the ubiquinone 10 was measured instead of mono-unsaturated fatty acid, the same result as the above was accepted. As a result of measuring change of the marker for oxidative stress generated by adding a radical initiator to human plasma, i.e., cholesterol ester hydroperoxide, the rise of the concentration was accepted. As a result of examining like the above whether this oxidant stress can be controlled by addition of a drug, it found out that the pyrazolone derivative shown by following formula (I) could control a rise of cholesterol ester hydroperoxide. These results as a marker for measuring the oxidant stress (oxidation obstacle) of organizations (brain etc.) at the same time it proves that the pyrazolone derivative shown by formula (I) controls the oxidant stress which rises at the time of symptoms, It is proved that mono-unsaturated fatty acid in plasma (namely, oleic acid (18:1) and palmitoleic acid (16:1)), the ubiquinone 10, and cholesterol ester hydroperoxide are useful. This invention is completed based on these knowledge.

. Namely, according to this invention, the pyrazolone derivative shown by following formula (I) or its salt permitted pharmacologically is included as an active principle. oxidant stress -- desirable -- mono-unsaturated fatty acid (namely, oleic acid (18:1) and/or palmitoleic acid (16:1)) in plasma. the ubiquinone 10 and/or cholesterol ester hydroperoxide -- the depressant of the ubiquinone 10 and/or cholesterol ester hydroperoxide is provided more preferably.



(I)

(Among a formula, R^1 expresses a hydrogen atom, an aryl group, the alkyl group of the carbon numbers 1-5, or the alkoxycarbonyl-alkyl group of the total carbon numbers 3-6, and;R^2) Express a hydrogen atom, an aryloxy group, an aryl sulfhydryl group, the alkyl group of the carbon numbers 1-5, or the hydroxyalkyl group of the carbon numbers 1-3, and;. Or R^1 and R^2, Express the alkylene group of the carbon numbers 3-5 jointly, and;R^3, A hydrogen atom, the alkyl group of the carbon numbers 1-5, the cycloalkyl group of the carbon numbers 5-7, The hydroxyalkyl group of the carbon numbers 1-3, benzyl, a naphthyl group, or unsubstituted, Or the alkyl group of the carbon numbers 1-5, the alkoxy group of the carbon numbers 1-5, the hydroxyalkyl group of the carbon numbers 1-3, The alkoxycarbonyl group of the total carbon numbers 2-5, the alkyl sulfhydryl group of the carbon numbers 1-3, The alkylamino group of the carbon numbers 1-4, the dialkylamino group of the total carbon numbers 2-8, The phenyl group replaced by 1-3 substituents which are chosen from the group which consists of a halogen atom, a trifluoromethyl group, a carboxyl group, a cyano group, a hydroxyl group, a nitro group, an amino group, and an acetamide group, and which are the same or are different is expressed.

Preferably, as an active principle, R^1 is an alkyl group of the carbon numbers 1-5, and R^2 is a hydrogen atom in formula (I), and R^3, The alkyl group of the carbon numbers 1-5, the alkoxy group of the carbon numbers 1-5, the hydroxyalkyl group of the carbon numbers 1-3, The alkoxycarbonyl group of the total carbon numbers 2-5, the alkyl sulfhydryl group of the carbon numbers 1-3, The alkylamino group of the carbon numbers 1-4, the dialkylamino group of the total carbon numbers 2-8, It is the phenyl group replaced by 1-3 substituents which are chosen from the group which consists of a halogen atom, a trifluoromethyl group, a carboxyl group, a cyano group, a hydroxyl group, a nitro group, an amino group, and an acetamide group, and which are the same or are different.

The oxidation stress inhibitor which contains 3-methyl-1-phenyl-2-pyrazoline 5-one or its salt permitted pharmacologically as an active principle is provided especially preferably.

Oxidation stress inhibitor of this invention can be preferably used as medicine for the therapy of the disease which induces, advances or gets worse by oxidant stress, and/or prevention. Preferably the disease which induces, advances or gets worse by oxidant stress Mono-unsaturated fatty acid, It is a disease accompanied by a rise of the ubiquinone 10 or cholesterol ester hydroperoxide, and is a disease accompanied by a rise of the ubiquinone 10 or cholesterol ester hydroperoxide more preferably. Oxidation stress inhibitor of this invention can control oxidant stress mono-unsaturated fatty acid in plasma, the ubiquinone 10 or cholesterol ester hydroperoxide, and by controlling the ubiquinone 10 or cholesterol ester hydroperoxide preferably.

According to another side of this invention, the method of controlling oxidant stress including medicating mammalian including Homo sapiens with the pyrazolone derivative pharmacologically shown by said an effective dose of formula (I)s or its salt permitted pharmacologically is provided.
According to another side of this invention, use of the pyrazolone derivative shown by said formula (I) in manufacture of oxidation stress inhibitor or its salt permitted pharmacologically is provided.
According to another side of this invention, as a marker Mono-unsaturated fatty acid in plasma, The measuring method of the ubiquinone 10 or cholesterol ester hydroperoxide, and the oxidant stress using the ubiquinone 10 or cholesterol ester hydroperoxide preferably is provided.
Preferably, mono-unsaturated fatty acid is oleic acid (18:1) and/or palmitoleic acid (16:1).
Preferably, measurement of mono-unsaturated fatty acid, the ubiquinone 10, or cholesterol ester hydroperoxide is performed with a liquid chromatography method.
According to another side of this invention, mono-unsaturated fatty acid, ubiquinone 10, or cholesterol ester hydroperoxide in a test subject's plasma, The content of the ubiquinone 10 or cholesterol ester hydroperoxide is measured preferably, and the clinical examination method analyzing or evaluating the symptoms of the disease which induces, advances or gets worse by oxidant stress from the measured value is provided.
Mono-unsaturated fatty acid in the plasma of the test subject who prescribed for the patient the drugs which are expected to have oxidant stress depressant action according to another side of this invention, The method of evaluating the validity of the oxidant stress depressant action in which the included drugs concerned have measuring the content of the ubiquinone 10 or cholesterol ester hydroperoxide is provided.
Preferably, drugs are a pyrazolone derivative shown by said formula (I), or its salt permitted pharmacologically, and more preferably, In formula (I), R^1 is an alkyl group of the carbon numbers 1-5, R^2 is a hydrogen atom and R^3 The alkyl group of the carbon numbers 1-5, The alkoxy group of the carbon numbers 1-5, the hydroxyalkyl group of the carbon numbers 1-3, The alkoxycarbonyl group of the total carbon numbers 2-5, the alkyl sulfhydryl group of the carbon numbers 1-3, The alkylamino group of the carbon numbers 1-4, the dialkylamino group of the total carbon numbers 2-8, It is the phenyl group replaced by 1-3 substituents which are chosen from the group which consists of a halogen atom, a trifluoromethyl group, a carboxyl group, a cyano group, a hydroxyl group, a nitro group, an amino group, and an acetamide group, and which are the same or are different. They are 3-methyl-1-phenyl-2-pyrazoline 5-one or its salt permitted pharmacologically especially preferably as the pyrazolone derivative shown by formula (I), or its salt permitted pharmacologically.
Mono-unsaturated fatty acid in the plasma of the patient expected to have trouble with his disease which induced, advanced or gets worse by oxidant stress according to another side of this invention, The content of the ubiquinone 10 or cholesterol ester hydroperoxide is measured, The symptoms of the disease which induces, advances or gets worse by oxidant stress from the measured value are analyzed or evaluated, As a result, the medicine prescribing for the patient the pyrazolone derivative shown by said formula (I) or its salt permitted pharmacologically to the patient judged to have trouble with one's disease which induced, advanced or gets worse by oxidant stress is provided. They are 3-methyl-1-phenyl-2-pyrazoline 5-one or its salt permitted pharmacologically especially preferably as the pyrazolone derivative shown by formula (I), or its salt permitted pharmacologically.

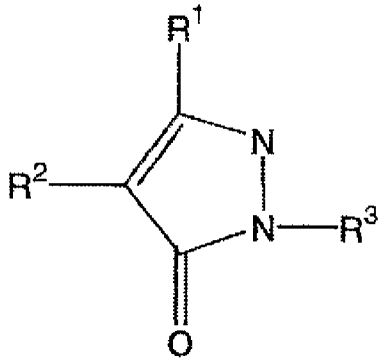
The best gestalt for inventing

Hereafter, an embodiment of the invention is described in detail.

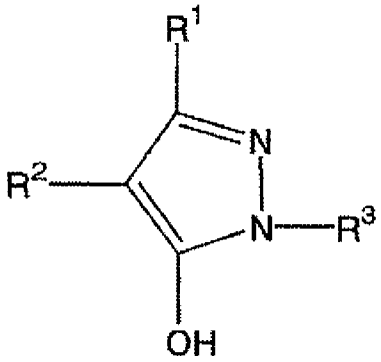
(I) Medicine of this invention

The medicine of this invention contains the pyrazolone derivative shown by formula (I) defined as this Description, or its salt permitted pharmacologically as an active principle.

The compound shown by formula (I) can also take the structure shown by the following formulas (I') or (I''). Therefore, the compound which takes a formula (I') or the structure of (I'') is also contained in the active principle of this invention.



(I')



(I'')

In formula (I), the phenyl group etc. which were replaced by substituents, such as a phenyl group and a methyl group, a butyl group, a methoxy group, a butoxy group, a chlorine atom, and a hydroxyl group, are mentioned as an aryl group in the definition of R^1.

As an alkyl group of the carbon numbers 1-5 in the definition of R^1, R^2, and R^3, a methyl group, an ethyl group, a propyl group, an isopropyl group, a butyl group, an isobutyl group, a sec-butyl group, a tert-butyl group, a pentyl group, etc. are mentioned.

As an alkoxycarbonyl-alkyl group of the total carbon numbers 3-6 in the definition of R^1, a methoxy carbonylmethyl group, an ethoxy carbonylmethyl group, a pro BOKISHI carbonylmethyl group, a methoxy carbonylethyl group, a carbomethoxypropyl group, etc. are mentioned.

As an aryloxy group in the definition of R^2, They are mentioned by a phenoxy group, p-methylphenoxy group, a p-methoxy phenoxy group, p-chloro phenoxy group, p-hydroxy phenoxy group, etc., and as an aryl sulfhydryl group, A phenylsulfhydryl group, p-methylphenyl sulfhydryl group, p-methoxypheny sulfhydryl group, p-chlorophenyl sulfhydryl group, p-hydroxyphenyl sulfhydryl group, etc. are mentioned.

As a hydroxyalkyl group of the carbon numbers 1-3 in the definition of R^2 and R^3, a hydroxymethyl group, 2-hydroxyethyl group, 3-hydroxypropyl group, etc. are mentioned. As a cycloalkyl group of the carbon numbers 5-7 in the definition of R^3, a cyclopentylic group, a cyclohexyl group, a cycloheptyl group, etc. are mentioned.

In the definition of R^3, as an alkoxy group of the carbon numbers 1-5 in the substituent of a phenyl group, They are mentioned by a methoxy group, an ethoxy basis, a pro BOKISHI group, an isopropanal BOKISHI

group, a butoxy group, pentyloxy group, etc., and as an alkoxycarbonyl group of the total carbon numbers 2-5, They are mentioned by a methoxycarbonyl group, an ethoxycarbonyl group, a pro BOKISHI carbonyl group, butoxycarbonyl group, etc., and as an alkyl sulfhydryl group of the carbon numbers 1-3, They are mentioned by a methylsulfhydryl group, an ethylsulfhydryl group, propylsulfhydryl group, etc., and as an alkylamino group of the carbon numbers 1-4, A methylamino group, an ethylamino group, a propylamino group, a butylamino group, etc. are mentioned, and a dimethylamino group, a diethylamino group, a dipropylamino group, a dibutylamino group, etc. are mentioned as a dialkylamino group of the total carbon numbers 2-8.

As an example of the compound of formula (I) used by this invention, the compound shown below is mentioned, for example.

3-methyl-1-phenyl-2-pyrazoline 5-one
3-methyl-1-(2-methylphenyl)-2-pyrazoline 5-one
3-methyl-1-(3-methylphenyl)-2-pyrazoline 5-one
3-methyl-1-(4-methylphenyl)-2-pyrazoline 5-one
3-methyl-1-(3,4-dimethylphenyl)-2-pyrazoline 5-one
1-(4-ethylphenyl)-3-methyl-2-pyrazoline 5-one
3-methyl-1-(4-propylphenyl)-2-pyrazoline 5-one
1-(4-buthylphenyl)-3-methyl-2-pyrazoline 5-one
1-(3-trifluoro methylphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-trifluoro methylphenyl)-3-methyl-2-pyrazoline 5-one
1-(2-methoxypheny)-3-methyl-2-pyrazoline 5-one
1-(3-methoxypheny)-3-methyl-2-pyrazoline 5-one
1-(4-methoxypheny)-3-methyl-2-pyrazoline 5-one
1-(3,4-dimethoxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-ethoxyphenyl)-3-methyl-2-pyrazoline 5-one
3-methyl-1-(4-pro BOKISHI phenyl)-2-pyrazoline 5-one
1-(4-butoxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(2-chlorophenyl)-3-methyl-2-pyrazoline 5-one
1-(3-chlorophenyl)-3-methyl-2-pyrazoline 5-one
1-(4-chlorophenyl)-3-methyl-2-pyrazoline 5-one
1-(3,4-dichlorophenyl)-3-methyl-2-pyrazoline 5-one
1-(4-bromophenyl)-3-methyl-2-pyrazoline 5-one
1-(4-fluorophenyl)-3-methyl-2-pyrazoline 5-one
1-(3-chloro-4-methylphenyl)-3-methyl-2-pyrazoline 5-one
1-(3-methylmercaptophenyl)-3-methyl-2-pyrazoline 5-one
1-(4-methylmercaptophenyl)-3-methyl-2-pyrazoline 5-one
4-(3-methyl-5-oxo 2-pyrazoline 1-yl) benzoic acid
1-(4-ethoxycarbonylphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-nitrophenyl)-3-methyl-2-pyrazoline 5-one
3-ethyl-1-phenyl-2-pyrazoline 5-one
1-phenyl-3-propyl-2-pyrazoline 5-one

1,3-diphenyl-2-pyrazoline 5-one
3-phenyl-1-(p-tolyl)-2-pyrazoline 5-one
1-(4-methoxypheny)-3-phenyl-2-pyrazoline 5-one
1-(4-chlorophenyl)-3-phenyl-2-pyrazoline 5-one
3,4-dimethyl- 1-phenyl-2-pyrazoline 5-one
4-isobutyl-3-methyl-1-phenyl-2-pyrazoline 5-one
4-(2-hydroxyethyl)-3-methyl-1-phenyl-2-pyrazoline 5-one
3-methyl-4-phenoxy-1-phenyl-2-pyrazoline 5-one
3-methyl-4-phenylmercapto-1-phenyl-2-pyrazoline 5-one
3,3',4,5,6,7-hexahydro 2-phenyl-2H-indazole 3-one
3-(ethoxy carbonylmethyl)-1-phenyl-2-pyrazoline 5-one
1-phenyl-2-pyrazoline 5-one
3-methyl-2-pyrazoline 5-one
1,3-dimethyl- 2-pyrazoline 5-one
1-ethyl-3-methyl-2-pyrazoline 5-one
1-butyl-3-methyl-2-pyrazoline 5-one
1-(2-hydronalium KIECHIRU)-3-methyl-2-pyrazoline 5-one
1-cyclohexyl-3-methyl-2-pyrazoline 5-one
1-benzyl-3-methyl-2-pyrazoline 5-one
1-(alpha-naphthyl)-3-methyl-2-pyrazoline 5-one
1-methyl-3-phenyl-2-pyrazoline 5-one
3-methyl-1-(4-methylphenyl)-2-pyrazoline 5-one
1-(4-buthylphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-methoxypheny)-3-methyl-2-pyrazoline 5-one
1-(4-butoxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-chlorophenyl)-3-methyl-2-pyrazoline 5-one
1-(4-hydroxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(3,4-dihydroxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(2-hydroxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(3-hydroxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-hydroxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(3,4-hydroxyphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-hydroxyphenyl)-3-phenyl-2-pyrazoline 5-one
1-(4-hydroxy methylphenyl)-3-methyl-2-pyrazoline 5-one
1-(4-aminophenyl)-3-methyl-2-pyrazoline 5-one
1-(4-methylamino phenyl)-3-methyl-2-pyrazoline 5-one
1-(4-ethylamino phenyl)-3-methyl-2-pyrazoline 5-one
1-(4-butylamino phenyl)-3-methyl-2-pyrazoline 5-one
1-(4-dimethylaminophenyl)-3-methyl-2-pyrazoline 5-one
1-(acetamide phenyl)-3-methyl-2-pyrazoline 5-one
1-(4-cyanophenyl)-3-methyl-2-pyrazoline 5-one

As a medicinal active principle of this invention, the salt permitted physiologically besides the compound of the isolation gestalt expressed with formula (I) may be used. As a salt permitted physiologically, salt; methanesulfonic acid with mineral acid, such as chloride, sulfuric acid, a hydrogen bromide salt, and phosphoric acid, P-toluenesulfonic acid, benzenesulfonic acid, acetic acid, glycolic acid, Glucuronic acid, maleic acid, fumaric acid, oxalic acid, ascorbic acid, A salt with organic acid, such as citrate, salicylic acid, nicotinic acid, and tartaric acid; Sodium, A salt with alkaline metals, such as potassium; Salt; ammonia with alkaline-earth metals, such as magnesium and calcium, A salt with amine, such as tris(hydroxymethyl) aminomethane, a N,N-bis(hydroxyethyl)piperazine, 2-amino-2-methyl-1-propanol, ethanolamine, N-methylglutamine, and L-glutamine, is mentioned. A salt with amino acid, such as a glycine, may be used.
As a medicinal active principle of this invention, the compound expressed with the above-mentioned formula (I), the hydrate of the salt permitted physiologically, the compound expressed with the above-mentioned formula (I), or its solvate of a salt permitted physiologically may be used. Although the kind in particular of organic solvent which forms solvate is not limited, methanol, ethanol, ether, dioxane, a tetrahydrofuran, etc. can be illustrated, for example. The compound expressed with the above-mentioned formula (I) may have one or more asymmetrical carbon according to the kind of substituent, and the stereoisomeric form of an optical isomer or a diastereoisomer may exist. As a medicinal active principle of this invention, the arbitrary mixtures of the stereoisomeric form of a pure gestalt and a stereoisomeric form, racemate, etc. may be used.
The compound of formula (I) used by this invention is a publicly known compound, for example, is indicated to JP,H5-31523,B, JP,H5-35128,B, etc.
The pyrazolone derivative shown by formula (I) used as an active principle by this invention, or its salt permitted pharmacologically, By controlling mono-unsaturated fatty acid, the ubiquinone 10, or cholesterol ester hydroperoxide in plasma, oxidant stress can be controlled and it can be used as medicine for the therapy of an oxidant stress disease, and/or prevention. Therefore, as an oxidant stress disease said on these Descriptions, the disease accompanied by a rise of mono-unsaturated fatty acid, the ubiquinone 10, or cholesterol ester hydroperoxide is preferred. As an example of an oxidant stress disease, although the following are mentioned, it is not limited to these.
A variety of peripheral circulatory bisturdance based on myocardial ischemias, such as a variety of encephalopathy, such as a cerebral blood vessel organization lesion accompanying the cerebral function fall, vascular dementia, and aging resulting from cerebrovascular disease, such as an ischemia disease or various kinds of diseases based on it, i.e., cerebral infarction, and cerebral apoplexy, or them, myocardial infarction, and cardiac insufficiency;
Endotoxin shock, a nephritis, diabetes mellitus, etc. which are based on alcoholic hepatitis, a retinopathy, retina ******, a cataract, the side-effects obstacle by radiation therapy, the cerebral edema, and an antigen toxin as other oxidant stress diseases are mentioned.
the dose of the compound of formula (I) used by this invention -- general -- parenteral administration -- 0.01 - 100 mg/kg and a day -- they are 0.1 - 10 mg/kg and a day preferably -- internal use -- 1-200mg/kg and a day -- they are 5 - 20 mg/kg and a day preferably. As for the above-mentioned dose, it is preferred to prescribe a medicine for the patient by 1 to 3 times per day. As for the above-mentioned dose, it is still more preferred to fluctuate suitably according to age, symptoms, and condition.
Although the compound expressed with the above-mentioned formula (I), its salt permitted physiologically,

those hydrates, or solvate may be prescribed for the patient as it is as medicine of this invention, generally, It is preferred to prepare and prescribe for the patient the medicinal composition containing the above-mentioned substance which is an active principle, and the additive permitted pharmacologically and in galenical pharmacy.

As an additive which can be permitted pharmacologically and in galenical pharmacy, an excipient, disintegrator or a collapse adjuvant, a binding material, lubricant, a coating agent, coloring matter, a diluent, a base, a resolvent or a solubilizing agent, an isotonizing agent, a pH regulator, a stabilizing agent, propellants, a binder, etc. can be used, for example.

In a medicinal composition suitable for internal use, as an additive, for example Grape sugar, Excipients, such as milk sugar, D-mannitol, starch, or crystalline cellulose; Carboxymethyl cellulose, Disintegrator or collapse adjuvants, such as starch or carboxymethyl-cellulose calcium; Hydroxypropylcellulose, Lubricant, such as binding material; magnesium stearate or talc, such as hydroxypropylmethylcellulose, a polyvinyl pyrrolidone, or gelatin; Hydroxypropylmethylcellulose, Coating agents, such as white soft sugar, a polyethylene glycol, or titanium oxide; bases, such as vaseline, a liquid paraffin, a polyethylene glycol, gelatin, kaolin, glycerin, purified water, or hard fat, can be used.

In a medicinal composition suitable for injection or a drop by drop titration. the aquosity of distilled water for injection, a physiological saline, propylene glycol, etc., or business -- the time -- dissolved type injections -- constituting -- obtaining -- a resolvent -- or -- a solubilizing agent --; -- grape sugar. Isotonizing agents, such as sodium chloride, D-mannitol, and glycerin; additives, such as pH regulators, such as inorganic acid, organic acid, an inorganic base, or an organic base, can be used.

The medicinal gestalt in particular of this invention is not limited, but can take available various gestalten to a person skilled in the art. A tablet, powder medicine, a granule, a ** gelatine capsule agent, suppositories, or trochiscus can be prepared, using the solid additive for pharmaceutical preparation for example as medicine suitable for internal use, and syrups, an emulsion, a ** gelatine capsule agent, etc. can be prepared using the liquefied additive for pharmaceutical preparation. Injections, drops, inhalations, suppositories, a percutaneous absorption agent, a permucosal absorbent, etc. can be prepared as medicine suitable for parenteral administration. The brain protection agent (drops) which makes the compound of above formula (I) an active principle, Since it is already used in clinical (a general name "edaravone", a trade name "Radicut": the Mitsubishi Pharma Corp. manufacture and sale), in the medicine of this invention, the above-mentioned commercial pharmaceutical preparation can be used as it is.

(II) A measuring method of the oxidant stress by this invention

In this invention, mono-unsaturated fatty acid, such as oleic acid (18:1) and palmitoleic acid (16:1), the ubiquinone 10 or cholesterol ester hydroperoxide -- oxidant stress is preferably measured by using the ubiquinone 10 or cholesterol ester hydroperoxide as a marker (index). The cholesterol ester hydroperoxide said on these Descriptions mainly means the hyperoxidation object of the fatty acid part of fatty acid ester of cholesterol. In this invention, plasma is extracted from the target living body and mono-unsaturated fatty acid, such as oleic acid (18:1) and palmitoleic acid (16:1), the ubiquinone 10, or cholesterol ester hydroperoxide which exists in the plasma is measured by various kinds of publicly known measurement techniques.

It is preferred to perform measurement of the quantity of mono-unsaturated fatty acid, such as oleic acid (18:1) and palmitoleic acid (16:1), the ubiquinone 10, or cholesterol ester hydroperoxide with liquid

chromatography methods, such as high speed liquid chromatography (HPLC).
Measurement of the quantity of mono-unsaturated fatty acid by high speed liquid chromatography, the ubiquinone 10, or cholesterol ester hydroperoxide can be performed in accordance with a conventional method, As a column, for example in measurement of mono-unsaturated fatty acid, Octyl column (3-micrometer, 3.3cmx4.6mm i.d., Supelco) +pkb-100 column (5 micrometers) 25cmx4.6mm i.d., Supelco, etc. can be used and acetonitrile / methanol / water =17.5/65.0 / 17.5 (v/v/v) can be used as a mobile phase, for example. The rate of flow can be considered as a part for 1.5-ml/, and column temperature can be set as 40 **. In the case of the ubiquinone 10, they are two guard columns () as a column. [Type Supelguard LC-ABZ and] 5 micrometers, 50x4.6mm i.d., and a Supelco+ analysis column (Type Supelcosil LC-8 or 5 micrometers) Can use 250x4.6mmi.d., a Supelco+ reduction column (Type RC-10-1, Irica), etc., and as a mobile phase, For example, methanol / tert-butyl alcohol =85 / 15 (v/v) (50mM sodium perchlorate is included) can be used, and it can be considered as a part for rate-of-flow:0.8-ml/. In the case of cholesterol ester hydroperoxide, as an analysis column (Type Beckman LC-8, 5 micrometers, 250x4.6mm i.d.) -- etc. -- it being used and as a mobile phase, For example, methanol / tert-butyl alcohol =19 / 1 (v/v) can be used, and it can be considered as a part for rate-of-flow:1.5-ml/of a chemiluminescence agent by rate-of-flow:1.0-ml/of a mobile phase, and can measure by the high speed liquid chromatography using a chemiluminescence method. However, these show an example of high speed liquid chromatography, and if they are persons skilled in the art, they can set up suitable conditions suitably.
When measuring mono-unsaturated fatty acid in this invention, the rate (%18:1) of oleic acid over the rate (%16:1) and the total free fatty acid of palmitoleic acid to the total free fatty acid is measured preferably.
The mono-unsaturated fatty acid obtained by the method mentioned above, such as oleic acid (18:1) and palmitoleic acid (16:1), And the quantity of the ubiquinone 10 and cholesterol ester hydroperoxide reflects the abundance of the active oxygen generated in in the living body, or a free radical.
Therefore, the state of oxidant stress is reflected.
Therefore, the state of the size of oxidant stress can be grasped by the size of the measured value. It is possible to analyze or evaluate the state of an oxidant stress disease effectively from the measured value.
As an oxidant stress disease, mono-unsaturated fatty acid, the ubiquinone 10, or cholesterol ester hydroperoxide, Although the disease accompanied by a rise of the ubiquinone 10 or cholesterol ester hydroperoxide is preferably preferred and it is as having carried out the account of this Description Nakagami as the example, it is not limited to them.
In this invention, mono-unsaturated fatty acid and the ubiquinone 10 which are used as a marker, and cholesterol ester hydroperoxide can be easily carried out from existing at a comparatively high rate in plasma using the HPLC art etc. which are adopted in the usual clinical laboratory test.
Mono-unsaturated fatty acid in the plasma of the test subject who prescribed for the patient the drugs which are expected to have oxidant stress depressant action according to this invention, The method of evaluating the validity of the oxidant stress depressant action in which the included drugs concerned have the ubiquinone 10 or cholesterol ester hydroperoxide, and measuring the content of the ubiquinone 10 or cholesterol ester hydroperoxide preferably is provided. Preferably, drugs are a pyrazolone derivative shown by said formula (I), or its salt permitted pharmacologically, and more preferably, In formula (I), R^1 is an alkyl group of the carbon numbers 1-5, R^2 is a hydrogen atom and R^3 The alkyl group of the carbon numbers 1-5,

The alkoxy group of the carbon numbers 1-5, the hydroxyalkyl group of the carbon numbers 1-3, The alkoxycarbonyl group of the total carbon numbers 2-5, the alkyl sulfhydryl group of the carbon numbers 1-3, The alkylamino group of the carbon numbers 1-4, the dialkylamino group of the total carbon numbers 2-8, It is the phenyl group replaced by 1-3 substituents which are chosen from the group which consists of a halogen atom, a trifluoromethyl group, a carboxyl group, a cyano group, a hydroxyl group, a nitro group, an amino group, and an acetamide group, and which are the same or are different. They are 3-methyl-1-phenyl-2-pyrazoline 5-one or its salt permitted pharmacologically especially preferably as the pyrazolone derivative shown by formula (I), or its salt permitted pharmacologically.

Mono-unsaturated fatty acid in the plasma of the patient expected to have trouble with his disease which induced, advanced or gets worse by oxidant stress furthermore according to this invention, The ubiquinone 10 or cholesterol ester hydroperoxide, The content of the ubiquinone 10 or cholesterol ester hydroperoxide is measured preferably, The symptoms of the disease which induces, advances or gets worse by oxidant stress from the measured value are analyzed or evaluated, As a result, the medicine prescribing for the patient the pyrazolone derivative shown by said formula (I) or its salt permitted pharmacologically to the patient judged to have trouble with one's disease which induced, advanced or gets worse by oxidant stress is provided. They are 3-methyl-1-phenyl-2-pyrazoline 5-one or its salt permitted pharmacologically especially preferably as the pyrazolone derivative shown by formula (I), or its salt permitted pharmacologically.

This invention is not limited by working example although the following working example explains this invention still more concretely.

EXAMPLE

A synthetic example: Composition of 3-methyl-1-phenyl-2-pyrazoline 5-one (edaravone is called hereafter) 13.0g of ethyl acetoacetate and 10.8 g of phenylhydrazine were added into 50 ml of ethanol, and flowing-back stirring was carried out for 3 hours. The precipitated crystal was ****(ed) after cooling reaction mixture radiationally, it recrystallized from ethanol, and the compound 11.3g of the title was obtained as a colorless crystal.

Yield 67%

Melting point 127.5-128.5 **

Working example 1 :

(Method)

1. Use animal

a 7-week old Crj:CD(SD) male rat (the time of arrival of goods -- weight: -- 197.8-228.8 g) Charles River Japan, Inc. was purchased 50 animals, observation and measurement of body weight of general status were performed during the quarantine habituation more than for five days, and after checking that it is normal and favorable weight transition is shown, the examination was presented by 8-week old. All the animals were bred in the rearing room set up in the temperature of 24**2 **, 55**10% of humidity, 13 air change rates/o'clock, and Lighting Sub-Division 12 hours (7:00 a.m. to 7:00 p.m.).

2. Composition of examination group

In order to prevent the variation in the cerebral infarction nest formation by a weight difference, a total of four groups of a group part opium poppy, physiological-salt-solution administration (a control group and a sham surgery (sham ope) group), and examined substance administration (a single-dose administration group and a repeated-dose administration group) were set up by the stratified continuation randomizing method based

on the weight of an operation day.

3. Production of MCA (middle cerebral artery) blockade recanalization model

The rat was fixed to the supine position after anesthesia induction by isoflurane inhalation 3%, and anesthesia was maintained by isoflurane inhalation 2%. In order to perform continuous intravenous drip infusion in the state of free moving, the catheter was detained in the femoral vein.

Cervix Masanaka skin incision was performed, the right-hand side common carotid artery, the external carotid artery, and the internal carotid artery were exposed, and the common carotid artery and the external carotid artery were litigated with the suture (No. 5). The nylon yarn (embolus) of No. 4 which carried out silicon coating (xantho PURENL, Bayer Yakuhin) beforehand, and was cut in length of 19 mm was inserted from the tee of an external carotid artery and an internal carotid artery, and MCA was blockaded. The embolus was extracted 2 hours after an MCA blockade, and recanalization of the blood flow of MCA was carried out. Neurological symptoms (crookedness of a forelimb) were observed 30 minutes after an MCA blockade, and the animal which cannot check crookedness of a forelimb was excepted from the examination. About the sham surgery group, it carried out based on this method to ligation of a right-hand side common carotid artery and an external carotid artery. In order to perform thermoregulation under operation, the probe for thermometers (PHYSITEMP INSTRUMENTS Inc.BAT-12) is inserted into the rectum, and the body temperature before and behind an operation was recorded. When decreased body temperature was seen, body temperature was maintained near 37 ** using the incandescent lamp.

4. Administration of drug

Continuous intravenous drip infusion (1st administration) for 30 minutes was performed for 3-methyl-1-phenyl-2-pyrazoline 5-one of immediately after [MCA blockade recanalization] 3 mg/kg by the capacity of 1.0 mL/body/h, and continuous intravenous drip infusion (2nd administration) for 30 minutes was again performed by the capacity after 6 hours. From the day following an operation. It will be an infusion pump (KD.) at 10:00 a.m. and 16:00 p.m. Continuous intravenous drip infusion for [bis die] 30 minutes was performed for 3-methyl-1-phenyl-2-pyrazoline 5-one of 3 mg/kg between 13 days of continuation by the capacity of 1.0 mL/body/h using SCIENTIFIC and 10 ream infusionpump 230. The concentration of administration liquid was computed based on the newest weight. The single-dose administration group prescribed the 1st 3-methyl-1-phenyl-2-pyrazoline 5-one of the operation day for the patient, and 2nd henceforth prescribed a physiological salt solution of the capacity for the patient. The control group and the sham surgery group were medicated with a physiological salt solution by the capacity.

5. Blood collecting

About 0.3 mL extraction of the blood was carried out from the subclavian vein using 1mL syringe which carried out heparin processing, centrifugality was carried out for 10 minutes and 3000 rpm of plasma was separated from 10:00 a.m. before an MCA blockade, after blockade recanalization, before 1, 2, 3, 5, 7, and the 1st administration start on the 10th, and on the 14th. the time of the sending after pouring plasma distributively 25microL every to four micro tubes and making it freeze with liquid nitrogen -- about -- cryopreservation was carried out at -80 **.

6. Separation fixed quantity of free fatty acid

It centrifuged, after being easy to add methanol of 200microl containing the margaric acid (13:0, internal standard) of 12.5microM to the plasma of 50microl and mixing (12,000 rpm, 3 minutes). Added cyano diester phosphate of dimethylformamide solution 50mul of 2mg/ml mono- dansylcadaverine, and 1microl after

removal under the nitrogen air current, the solvent of 50micro of supernatant liquid I was made to react for 20 minutes in a dark place, and free fatty acid was formed into fluorescence derivation.
5micro of reaction mixture I was poured into high speed liquid chromatography, and the amount of total fatty acid and fatty acid composition were investigated. The conditions of high speed liquid chromatography are as follows.
Column: Octyl column (3-micrometer, 3.3cmx4.6mm i.d., Supelco) +pkb-100 column (5 micrometers, 25cmx4.6mm i.d., Supelco)
Mobile phase: Acetonitrile / methanol / water =17.5/65.0/17.5 (v/v/v)
The rate of flow: A part for 1.5-ml/
Column temperature: 40 **
Fluorescence-detector excited wavelengths: 320 nm
Fluorescence-detector fluorescence wavelength: 520 nm
(Result - consideration)
A result is shown in drawing 1 and drawing 2.
Drawing 1 is a graph which shows the change with time of the rate of palmitoleic acid (16:1) in plasma. The rate of palmitoleic acid (16:1) over the total free fatty acid is defined as %16:1. In drawing 1, the percentage (%) of change is shown on the vertical axis of a graph on the basis of the value of %16:1 in the 0th day, and days are shown on the horizontal axis of a graph.
Drawing 2 is a graph which shows the change with time of the rate of oleic acid (18:1) in plasma. The rate of oleic acid over the total free fatty acid is defined as %18:1. The percentage (%) of change is shown on the vertical axis of a graph on the basis of the value of %18:1 in the 0th day, and days are shown on the horizontal axis of a graph.
In the rat of the sham surgery group, palmitoleic acid (16:1) and oleic acid (18:1) were comparatively alike, and a big change was not seen. However, in the rat of the control group, the rate of palmitoleic acid (16:1) and oleic acid (18:1) rose [after / MCA blockade-recanalization] intentionally to the backward one on one to the 5th, and oxidant stress sthenia in the meantime was suggested. On the other hand, in the group which prescribed 3-methyl-1-phenyl-2-pyrazoline 5-one for the patient, the rate of palmitoleic acid (16:1) and oleic acid (18:1) hardly changed so that similarly to a sham surgery group, but it was intentionally controlled as compared with the control group.
The above-mentioned result suggests protecting a brain, when 3-methyl-1-phenyl-2-pyrazoline 5-one eliminates effectively the radical which increases after brain ischemia-recanalization and inhibits lipid peroxidation.
Working example 2: A separation fixed quantity of the ubiquinone 10 (CoQ-10)
(Method)
Composition of a use animal and an examination group, production of an MCA blockade recanalization model, administration of a drug, and blood collecting were performed like working example 1. A separation fixed quantity of the ubiquinone 10 (CoQ-10) was performed as follows.
It centrifuged, after being easy to add the cold hexane of cold methanol of 250microl, and 500microl to the plasma of 50microl and mixing (for 10,000 g and 3 minutes, 4 **). The hexane layer (equivalent to the plasma of 0.5microl) of 5microl was promptly poured into high speed liquid chromatography, and the amount of ubiquinone 10 (CoQ-10) was investigated. The conditions of high speed liquid chromatography are as

follows.
Column: Two guard columns () [Type Supelguard LC-ABZ and] 5 micrometers, 50x4.6mm i.d., a Supelco+ analysis column (Type Supelcosil LC-8, 5-micrometer, 250x4.6mm i.d., Supelco) + reduction column (Type RC-10-1, Irica)
Moving bed: Methanol / tert-butyl alcohol =85/15 (v/v) (50mM sodium perchlorate is included)
The rate of flow: A part for 0.8-ml/
detection: -- electrochemical detector operation voltage: -- +600 mV
(Result)
A result is shown in drawing 3.
Drawing 3 is a graph which shows the change with time of the rate of the ubiquinone 10 in plasma. In drawing 3, the percentage (%) of change is shown on the vertical axis of a graph on the basis of the value of the ubiquinone 10 in the 0th day, and days are shown on the horizontal axis of a graph.
In the rat of the sham surgery group, the ubiquinone 10 was comparatively alike and a big change was not seen. However, in the rat of the control group, the rate of the after [MCA blockade-recanalization] ubiquinone 10 rose, and oxidant stress sthenia in the meantime was suggested. On the other hand, in the group which prescribed 3-methyl-1-phenyl-2-pyrazoline 5-one for the patient, the rate of the ubiquinone 10 hardly changed so that similarly to a sham surgery group. Since the ubiquinone 10 was not detected by measurement of the ubiquinone 10 on the 5th in two examples of a sham surgery group, and all the examples except one repeated-dose administration group, the data on the 5th was deleted in drawing 3.
Working example 3: Measurement of cholesterol ester hydroperoxide (CE-OOH) (method)
According to the method stated to working example 1, plasma was separated from the blood provided by healthy 40 years-old generation male. The 2,2'-azobis (2,4-dimethyl- valeronitrile) (AMVN) (it purchases from Wako Pure Chem) which is a radical initiator was added to 2 ml of this plasma so that AMVN concentration might serve as 10mM. Edaravone was further added to the edaravone administration group so that edaravone concentration might be set to 50microM.
The cocktail was kept at 37 ** and the amount of cholesterol ester hydroperoxide contained in a cocktail in 4, 5, and 6 hours was measured with the high performance chromatography using an isoluminol chemiluminescence method. The passage of the following [measuring condition].
Preparation of a chemiluminescence agent
177.2-mg isoluminol (it purchases from a sigma company) and 5 mg of micro peroxidase (it purchases from a sigma company) were dissolved into the 500-ml mixed liquor of methanol and 500 ml of borate salt buffer solution (pH 10), and the chemiluminescence agent was prepared.
The rate of flow
mobile phase (methanol / t-butanol =19/1 (v/v)): -- a part for 1.0-ml/-- chemiluminescence agent: -- a part for 1.5-ml/
Column
Analysis column: Type Beckman LC-8, 5 micrometers, 250x4.6mm i.d.
(Result)
A result is shown in drawing 4.
Drawing 4 is a graph which shows the change with time of the cholesterol ester hydroperoxide concentration contained in a cocktail. In drawing 4, cholesterol ester hydroperoxide concentration is shown on the vertical

axis of a graph, and lapsed time is shown on the horizontal axis of a graph. In the group which added 3-methyl-1-phenyl-2-pyrazoline 5-one (drugs) after after 5-hour progress, the increase in cholesterol ester hydroperoxide concentration was controlled.

Possibility of industrial use

the medicine of this invention -- various kinds of oxidant stress diseases (for example, an ischemia disease or various kinds of diseases based on it.) That is, it is useful as prevention and treating agents, such as hepatopathies, such as a variety of peripheral circulatory bisturdance based on myocardial ischemias, such as a variety of encephalopathy, such as a cerebral blood vessel organization lesion accompanying the cerebral function fall, vascular dementia, and aging resulting from cerebrovascular disease, such as cerebral infarction and cerebral apoplexy, or them, myocardial infarction, and cardiac insufficiency, and diabetes mellitus.

Furthermore by this invention, the new method for measuring oxidant stress exactly and quantitatively is provided. The method of this invention can measure blood etc. as a preparation, without carrying out invasion of the organization. Let content of mono-unsaturated fatty acid in plasma, the ubiquinone 10, or cholesterol ester hydroperoxide be a marker in this invention.

Therefore, the method of evaluating the validity of the oxidant stress depressant action which the pyrazolone derivative which has a fixed structure written in this Description has is provided.

According to the method of this invention, the validity as drugs of the pyrazolone derivative concerned can be evaluated exactly and easily.

All the contents given in the Description of the application for patent 2001-275466 which is application used as the foundation of the right of priority which this application claims, and the application for patent 2001-275467 shall be incorporated by citation into this Description as a part of indication of this Description.

[Brief Description of the Drawings]

Drawing 1 is a graph which shows the change with time of the rate of palmitoleic acid (16:1) in plasma.

Drawing 2 is a graph which shows the change with time of the rate of oleic acid (18:1) in plasma.

Drawing 3 is a graph which shows the change with time of the rate of the ubiquinone 10 in plasma.

Drawing 4 is a graph which shows the change with time of the rate of the cholesterol ester hydroperoxide in plasma.

[Translation done.]